

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICATION FOR PATENT OF:

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FOR:

**HUMAN ORPHAN G PROTEIN-COUPLED
RECEPTORS**

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HUMAN ORPHAN G PROTEIN-COUPLED RECEPTORS

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This patent document claims priority benefit of each of the following applications, all filed with the United States Patent and Trademark Office via U.S. Express Mail on the indicated filing dates: U.S. Provisional Number 60/121,852, filed; February 26, 1999
5 claiming the benefit of U.S. Provisional Number 60/109,213, filed November 20, 1998; U.S. Provisional Number 60/120,416, filed February 16, 1999; U.S. Provisional Number 60/123,946, filed March 12, 1999; U.S. Provisional Number 60/123,949, filed March 12, 1999; U.S. Provisional Number 60/136,436, filed May 28, 1999; U.S. Provisional Number 60/136,439, filed May 28, 1999; U.S. Provisional Number 60/136,567, filed May
10 28, 1999; U.S. Provisional Number 60/137,127, filed May 28, 1999; U.S. Provisional Number 60/137,131, filed May 28, 1999; U.S. Provisional Number 141,448, filed June 29, 1999 claiming priority from U.S. Provisional Number 60/136,437, filed May 28, 1999; U.S. Provisional Number _____ (Arena Pharmaceuticals, Inc. docket number CHN10-1), filed September 29, 1999; U.S. Provisional Number 60/156,333, filed
15 September 29, 1999; U.S. Provisional Number 60/156,555, filed September 29, 1999; U.S. Provisional Number 60/156,634, filed September 29, 1999; U.S. Provisional Number _____ (Arena Pharmaceuticals, Inc. docket number RUP6-1), filed October 1, 1999; U.S. Provisional Number _____ (Arena Pharmaceuticals, Inc. docket number

RUP7-1), filed October 1, 1999; U.S. Provisional Number _____ (Arena Pharmaceuticals, Inc. docket number CHN6-1), filed October 1, 1999; U.S. Provisional Number _____ (Arena Pharmaceuticals, Inc. docket number RUP5-1), filed October 1, 1999; U.S. Provisional Number _____ (Arena Pharmaceuticals, Inc. docket number 5 CHN9-1), filed October 1, 1999. This patent document is related to U.S. Serial Number 09/170,496 filed October 13, 1999, and U.S. Serial Number unknown (Woodcock Washburn Kurtz Mackiewicz & Norris, LLP docket number AREN-0054) filed on October 12, 1999 (via U.S. Express Mail) both being incorporated herein by reference. This patent document also is related to U.S. Serial No. 09/364,425; filed July 30, 1999, 10 which is incorporated by reference in its entirety. Each of the foregoing applications are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

The invention disclosed in this patent document relates to transmembrane receptors, and more particularly to endogenous, orphan, human G protein-coupled receptors 15 ("GPCRs").

BACKGROUND OF THE INVENTION

Although a number of receptor classes exist in humans, by far the most abundant and therapeutically relevant is represented by the G protein-coupled receptor (GPCR or GPCRs) class. It is estimated that there are some 100,000 genes within the human genome, and of 20 these, approximately 2% or 2,000 genes, are estimated to code for GPCRs. Receptors, including GPCRs, for which the endogenous ligand has been identified are referred to as "known" receptors, while receptors for which the endogenous ligand has not been identified

are referred to as "orphan" receptors. GPCRs represent an important area for the development of pharmaceutical products: from approximately 20 of the 100 known GPCRs, 60% of all prescription pharmaceuticals have been developed. This distinction is not merely semantic, particularly in the case of GPCRs. Thus, the orphan GPCRs are to the pharmaceutical industry what gold was to California in the late 19th century - an opportunity to drive growth, expansion, enhancement and development.

GPCRs share a common structural motif. All these receptors have seven sequences of between 22 to 24 hydrophobic amino acids that form seven alpha helices, each of which spans the membrane (each span is identified by number, *i.e.*, transmembrane-1 (TM-1), transmembrane-2 (TM-2), etc.). The transmembrane helices are joined by strands of amino acids between transmembrane-2 and transmembrane-3, transmembrane-4 and transmembrane-5, and transmembrane-6 and transmembrane-7 on the exterior, or "extracellular" side, of the cell membrane (these are referred to as "extracellular" regions 1, 2 and 3 (EC-1, EC-2 and EC-3), respectively). The transmembrane helices are also joined by strands of amino acids between transmembrane-1 and transmembrane-2, transmembrane-3 and transmembrane-4, and transmembrane-5 and transmembrane-6 on the interior, or "intracellular" side, of the cell membrane (these are referred to as "intracellular" regions 1, 2 and 3 (IC-1, IC-2 and IC-3), respectively). The "carboxy" ("C") terminus of the receptor lies in the intracellular space within the cell, and the "amino" ("N") terminus of the receptor lies in the extracellular space outside of the cell.

Generally, when an endogenous ligand binds with the receptor (often referred to as "activation" of the receptor), there is a change in the conformation of the intracellular region that allows for coupling between the intracellular region and an intracellular "G-protein." It

has been reported that GPCRs are "promiscuous" with respect to G proteins, *i.e.*, that a GPCR can interact with more than one G protein. *See*, Kenakin, T., 43 *Life Sciences* 1095 (1988). Although other G proteins exist, currently, Gq, Gs, Gi, and Go are G proteins that have been identified. Endogenous ligand-activated GPCR coupling with the G-protein begins a signaling cascade process (referred to as "signal transduction"). Under normal conditions, signal transduction ultimately results in cellular activation or cellular inhibition. It is thought that the IC-3 loop as well as the carboxy terminus of the receptor interact with the G protein.

Under physiological conditions, GPCRs exist in the cell membrane in equilibrium between two different conformations: an "inactive" state and an "active" state. A receptor in an inactive state is unable to link to the intracellular signaling transduction pathway to produce a biological response. Changing the receptor conformation to the active state allows linkage to the transduction pathway (via the G-protein) and produces a biological response. A receptor may be stabilized in an active state by an endogenous ligand or a compound such as a drug.

SUMMARY OF THE INVENTION

Disclosed herein are human endogenous orphan G protein-coupled receptors.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B provide reference "grids" for certain dot-blot analyses provided herein (*see also*, Figure 2A and 2B, respectively).

Figures 2A and 2B provide reproductions of the results of certain dot-blot analyses resulting from hCHN3 and hCHN8, respectively (*see also*, Figures 1A and 1B, respectively).

Figure 3 provides a reproduction of the results of RT-PCR analysis of hRUP3.

Figure 4 provides a reproduction of the results of RT-PCR analysis of hRUP4.

Figure 5 provides a reproduction of the results of RT-PCR analysis of hRUP6.

DETAILED DESCRIPTION

The scientific literature that has evolved around receptors has adopted a number of terms to refer to ligands having various effects on receptors. For clarity and consistency, the following definitions will be used throughout this patent document. To the extent that these definitions conflict with other definitions for these terms, the following definitions shall control:

AMINO ACID ABBREVIATIONS used herein are set out in Table 1:

TABLE 1		
10	ALANINE	ALA
	ARGININE	ARG
	ASPARAGINE	ASN
	ASPARTIC ACID	ASP
15	CYSTEINE	CYS
	GLUTAMIC ACID	GLU
	GLUTAMINE	GLN
	GLYCINE	GLY
	HISTIDINE	HIS
20	ISOLEUCINE	ILE
	LEUCINE	LEU
	LYSINE	LYS
	METHIONINE	MET
	PHENYLALANINE	PHE
25	PROLINE	PRO
	SERINE	SER
	THREONINE	THR
	TRYPTOPHAN	TRP
	TYROSINE	TYR
30	VALINE	VAL

COMPOSITION means a material comprising at least one component.

ENDOGENOUS shall mean a material that a mammal naturally produces. ENDOGENOUS in reference to, for example and not limitation, the term "receptor," shall mean that which is naturally produced by a mammal (for example, and not limitation, a

human) or a virus. By contrast, the term **NON-ENDOGENOUS** in this context shall mean that which is not naturally produced by a mammal (for example, and not limitation, a human) or a virus.

HOST CELL shall mean a cell capable of having a Plasmid and/or Vector incorporated therein. In the case of a prokaryotic Host Cell, a Plasmid is typically replicated as an autonomous molecule as the Host Cell replicates (generally, the Plasmid is thereafter isolated for introduction into a eukaryotic Host Cell); in the case of a eukaryotic Host Cell, a Plasmid is integrated into the cellular DNA of the Host Cell such that when the eukaryotic Host Cell replicates, the Plasmid replicates. Preferably, for the purposes of the invention disclosed herein, the Host Cell is eukaryotic, more preferably, mammalian, and most preferably selected from the group consisting of 293, 293T and COS-7 cells.

LIGAND shall mean an endogenous, naturally occurring molecule specific for an endogenous, naturally occurring receptor.

NON-ORPHAN RECEPTOR shall mean an endogenous naturally occurring molecule specific for an endogenous naturally occurring ligand wherein the binding of a ligand to a receptor activates an intracellular signaling pathway.

ORPHAN RECEPTOR shall mean an endogenous receptor for which the endogenous ligand specific for that receptor has not been identified or is not known.

PLASMID shall mean the combination of a Vector and cDNA. Generally, a Plasmid is introduced into a Host Cell for the purposes of replication and/or expression of the cDNA as a protein.

VECTOR in reference to cDNA shall mean a circular DNA capable of incorporating at least one cDNA and capable of incorporation into a Host Cell.

The order of the following sections is set forth for presentational efficiency and is not intended, nor should be construed, as a limitation on the disclosure or the claims to follow.

Identification of Human GPCRs

5 The efforts of the Human Genome project have led to the identification of a plethora of information regarding nucleic acid sequences located within the human genome; it has been the case in this endeavor that genetic sequence information has been made available without an understanding or recognition as to whether or not any particular genomic sequence does or may contain open-reading frame information that translate human proteins.

10 Several methods of identifying nucleic acid sequences within the human genome are within the purview of those having ordinary skill in the art. For example, and not limitation, a variety of GPCRs, disclosed herein, were discovered by reviewing the GenBank™ database, while other GPCRs were discovered by utilizing a nucleic acid sequence of a GPCR, previously sequenced, to conduct a BLAST™ search of the EST database. Table A, below,

15 lists the disclosed endogenous orphan GPCRs along with a GPCR's respective homologous GPCR:

TABLE A

	Disclosed	Accession	Open Reading	Per Cent	Reference To
	Human	Number	Frame	Homology	Homologous
20	Orphan	Identified	(Base Pairs)	To Designated	GPCR
	GPCRs			GPCR	(Accession No.)
	hARE-3	AL033379	1,260 bp	52.3% LPA-R	U92642
	hARE-4	AC006087	1,119 bp	36% P2Y5	AF000546

	hARE-5	AC006255	1,104 bp	32% <i>Oryzias</i>	D43633
				<i>latipes</i>	
	hGPR27	AA775870	1,128 bp		
	hARE-1	AI090920	999 bp	43%	D13626
				KIAA0001	
	hARE-2	AA359504	1,122 bp	53% GPR27	
5	hPPR1	H67224	1,053 bp	39% EBI1	L31581
	hG2A	AA754702	1,113 bp	31% GPR4	L36148
	hRUP3	AL035423	1,005 bp	30%	2133653
				<i>Drosophila</i>	
				<i>melanogaster</i>	
	hRUP4	AI307658	1,296 bp	32% pNPGPR	NP_004876
				28% and 29 %	AAC41276
				<i>Zebra fish</i> Ya	and
				and Yb,	AAB94616
				respectively	
	hRUP5	AC005849	1,413 bp	25% DEZ	Q99788
				23% FMLPR	P21462
10	hRUP6	AC005871	1,245 bp	48% GPR66	NP_006047
	hRUP7	AC007922	1,173 bp	43% H3R	AF140538
	hCHN3	EST 36581	1,113 bp	53% GPR27	
	hCHN4	AA804531	1,077 bp	32% thrombin	4503637
	hCHN6	EST 2134670	1,503 bp	36% edg-1	NP_001391
15	hCHN8	EST 764455	1,029 bp	47%	D13626
				KIAA0001	
	hCHN9	EST 1541536	1,077 bp	41% LTB4R	NM_000752
	hCHN10	EST 1365839	1,055 bp	35% P2Y	NM_002563

Receptor homology is useful in terms of gaining an appreciation of a role of the disclosed receptors within the human body. Additionally, such homology can provide insight 20 as to possible endogenous ligand(s) that may be natural activators for the disclosed orphan GPCRs.

B. Receptor Screening

Techniques have become more readily available over the past few years for

endogenous-ligand identification (this, primarily, for the purpose of providing a means of conducting receptor-binding assays that require a receptor's endogenous ligand) because the traditional study of receptors has always proceeded from the a priori assumption (historically based) that the endogenous ligand must first be identified before discovery could proceed to find antagonists and other molecules that could affect the receptor. Even in cases where an antagonist might have been known first, the search immediately extended to looking for the endogenous ligand. This mode of thinking has persisted in receptor research even after the discovery of constitutively activated receptors. What has not been heretofore recognized is that it is the active state of the receptor that is most useful for discovering agonists, partial agonists, and inverse agonists of the receptor. For those diseases which result from an overly active receptor or an under-active receptor, what is desired in a therapeutic drug is a compound which acts to diminish the active state of a receptor or enhance the activity of the receptor, respectively, not necessarily a drug which is an antagonist to the endogenous ligand. This is because a compound that reduces or enhances the activity of the active receptor state need not bind at the same site as the endogenous ligand. Thus, as taught by a method of this invention, any search for therapeutic compounds should start by screening compounds against the ligand-independent active state.

As is known in the art, GPCRs can be "active" in their endogenous state even without the binding of the receptor's endogenous ligand thereto. Such naturally-active receptors can be screened for the direct identification (*i.e.*, without the need for the receptor's endogenous ligand) of, in particular, inverse agonists. Alternatively, the receptor can be "activated" via, *e.g.*, mutation of the receptor to establish a non-endogenous version of the receptor that is active in the absence of the receptor's endogenous ligand.

Screening candidate compounds against an endogenous or non-endogenous, constitutively activated version of the human orphan GPCRs disclosed herein can provide for the direct identification of candidate compounds which act at this cell surface receptor, without requiring use of the receptor's endogenous ligand. By determining areas within the body where the endogenous version of human GPCRs disclosed herein is expressed and/or over-expressed, it is possible to determine related disease/disorder states which are associated with the expression and/or over-expression of the receptor; such an approach is disclosed in this patent document.

With respect to creation of a mutation that may evidence constitutive activation of human orphan GPCRs disclosed herein is based upon the distance from the proline residue at which is presumed to be located within TM6 of the GPCR typically nears the TM6/IC3 interface (such proline residue appears to be quite conserved). By mutating the amino acid residue located 16 amino acid residues from this residue (presumably located in the IC3 region of the receptor) to, most preferably, a lysine residue, such activation may be obtained. Other amino acid residues may be useful in the mutation at this position to achieve this objective.

C. Disease/Disorder Identification and/or Selection

Preferably, the DNA sequence of the human orphan GPCR can be used to make a probe for (a) dot-blot analysis against tissue-mRNA, and/or (b) RT-PCR identification of the expression of the receptor in tissue samples. The presence of a receptor in a tissue source, or a diseased tissue, or the presence of the receptor at elevated concentrations in a diseased tissue compared to a normal tissue, can be preferably utilized to identify a correlation with a treatment regimen, including but not limited to, a disease associated

with that disease. Receptors can equally well be localized to regions of organs by this technique. Based on the known functions of the specific tissues to which the receptor is localized, the putative functional role of the receptor can be deduced.

D. Screening of Candidate Compounds

5 1. Generic GPCR screening assay techniques

When a G protein receptor becomes constitutively active (i.e., active in the absence of endogenous ligand binding thereto), it binds to a G protein (e.g., Gq, Gs, Gi, Go) and stimulates the binding of GTP to the G protein. The G protein then acts as a GTPase and slowly hydrolyzes the GTP to GDP, whereby the receptor, under normal conditions, becomes
10 deactivated. However, constitutively activated receptors continue to exchange GDP to GTP.

A non-hydrolyzable analog of GTP, [³⁵S]GTPγS, can be used to monitor enhanced binding to membranes which express constitutively activated receptors. It is reported that [³⁵S]GTPγS can be used to monitor G protein coupling to membranes in the absence and presence of ligand. An example of this monitoring, among other examples well-known and
15 available to those in the art, was reported by Traynor and Nahorski in 1995. The preferred use of this assay system is for initial screening of candidate compounds because the system is generically applicable to all G protein-coupled receptors regardless of the particular G protein that interacts with the intracellular domain of the receptor.

2. Specific GPCR screening assay techniques

20 Once candidate compounds are identified using the "generic" G protein-coupled receptor assay (i.e., an assay to select compounds that are agonists, partial agonists, or inverse agonists), further screening to confirm that the compounds have interacted at the receptor site is preferred. For example, a compound identified by the "generic" assay may not bind to the

receptor, but may instead merely "uncouple" the G protein from the intracellular domain.

a. Gs and Gi.

Gs stimulates the enzyme adenylyl cyclase. Gi (and Go), on the other hand, inhibit this enzyme. Adenylyl cyclase catalyzes the conversion of ATP to cAMP; thus, 5 constitutively activated GPCRs that couple the Gs protein are associated with increased cellular levels of cAMP. On the other hand, constitutively activated GPCRs that couple the Gi (or Go) protein are associated with decreased cellular levels of cAMP. *See, generally, "Indirect Mechanisms of Synaptic Transmission," Chpt. 8, From Neuron To Brain (3rd Ed.)* Nichols, J.G. et al eds. Sinauer Associates, Inc. (1992). Thus, assays that detect cAMP can 10 be utilized to determine if a candidate compound is, *e.g.*, an inverse agonist to the receptor (*i.e.*, such a compound would decrease the levels of cAMP). A variety of approaches known in the art for measuring cAMP can be utilized; a most preferred approach relies upon the use of anti-cAMP antibodies in an ELISA-based format. Another type of assay that can be utilized is a whole cell second messenger reporter system assay. Promoters on genes drive 15 the expression of the proteins that a particular gene encodes. Cyclic AMP drives gene expression by promoting the binding of a cAMP-responsive DNA binding protein or transcription factor (CREB) which then binds to the promoter at specific sites called cAMP response elements and drives the expression of the gene. Reporter systems can be constructed which have a promoter containing multiple cAMP response elements before the reporter 20 gene, *e.g.*, β -galactosidase or luciferase. Thus, a constitutively activated Gs-linked receptor causes the accumulation of cAMP that then activates the gene and expression of the reporter protein. The reporter protein such as β -galactosidase or luciferase can then be detected using standard biochemical assays (Chen et al. 1995).

Go and Gq.

Gq and Go are associated with activation of the enzyme phospholipase C, which in turn hydrolyzes the phospholipid PIP_2 , releasing two intracellular messengers: 5 diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). Increased accumulation of IP_3 is associated with activation of Gq- and Go-associated receptors. *See, generally*, "Indirect Mechanisms of Synaptic Transmission," Chpt. 8, From Neuron To Brain (3rd Ed.) Nichols, J.G. et al eds. Sinauer Associates, Inc. (1992). Assays that detect IP_3 accumulation can be utilized to determine if a candidate compound is, *e.g.*, an inverse agonist to a Gq- or Go- 10 associated receptor (*i.e.*, such a compound would decrease the levels of IP_3). Gq-associated receptors can also be examined using an AP1 reporter assay in that Gq-dependent phospholipase C causes activation of genes containing AP1 elements; thus, activated Gq-associated receptors will evidence an increase in the expression of such genes, whereby inverse agonists thereto will evidence a decrease in such expression, and agonists will 15 evidence an increase in such expression. Commercially available assays for such detection are available.

3. GPCR Fusion Protein

The use of an endogenous, constitutively activated orphan GPCR, or a non- 20 endogenous, constitutively activated orphan GPCR, for screening of candidate compounds for the direct identification of inverse agonists, agonists and partial agonists provides a unique challenge in that, by definition, the receptor is active even in the absence of an endogenous ligand bound thereto. Thus, it is often useful that an approach be utilized that can enhance the signal obtained by the activated receptor. A preferred approach is the use of a GPCR Fusion Protein.

Generally, once it is determined that a GPCR is or has been constitutively activated, using the assay techniques set forth above (as well as others), it is possible to determine the predominant G protein that couples with the endogenous GPCR. Coupling of the G protein to the GPCR provides a signaling pathway that can be assessed. Because it is most preferred that screening take place by use of a mammalian expression system, such a system will be expected to have endogenous G protein therein. Thus, by definition, in such a system, the constitutively activated orphan GPCR will continuously signal. In this regard, it is preferred that this signal be enhanced such that in the presence of, *e.g.*, an inverse agonist to the receptor, it is more likely that it will be able to more readily differentiate, particularly in the context of screening, between the receptor when it is contacted with the inverse agonist.

The GPCR Fusion Protein is intended to enhance the efficacy of G protein coupling with the GPCR. The GPCR Fusion Protein is preferred for screening with a non-endogenous, constitutively activated GPCR because such an approach increases the signal that is most preferably utilized in such screening techniques, although the GPCR Fusion Protein can also be (and preferably is) used with an endogenous, constitutively activated GPCR. This is important in facilitating a significant "signal to noise" ratio; such a significant ratio is import preferred for the screening of candidate compounds as disclosed herein.

The construction of a construct useful for expression of a GPCR Fusion Protein is within the purview of those having ordinary skill in the art. Commercially available expression vectors and systems offer a variety of approaches that can fit the particular needs of an investigator. The criteria of importance for such a GPCR Fusion Protein construct is that the GPCR sequence and the G protein sequence both be in-frame (preferably, the sequence for the GPCR is upstream of the G protein sequence) and that the "stop" codon of

the GPCR must be deleted or replaced such that upon expression of the GPCR, the G protein can also be expressed. The GPCR can be linked directly to the G protein, or there can be spacer residues between the two (preferably, no more than about 12, although this number can be readily ascertained by one of ordinary skill in the art). We have a preference (based upon convenience) of use of a spacer in that some restriction sites that are not used will, effectively, upon expression, become a spacer. Most preferably, the G protein that couples to the GPCR will have been identified prior to the creation of the GPCR Fusion Protein construct. Because there are only a few G proteins that have been identified, it is preferred that a construct comprising the sequence of the G protein (*i.e.*, a universal G protein construct) be available for insertion of an endogenous GPCR sequence therein; this provides for efficiency in the context of large-scale screening of a variety of different endogenous GPCRs having different sequences.

E. Other Utility

Although a preferred use of the human orphan GPCRs disclosed herein may be for the direct identification of candidate compounds as inverse agonists, agonists or partial agonists (preferably for use as pharmaceutical agents), these versions of human GPCRs can also be utilized in research settings. For example, *in vitro* and *in vivo* systems incorporating GPCRs can be utilized to further elucidate and understand the roles these receptors play in the human condition, both normal and diseased, as well as understanding the role of constitutive activation as it applies to understanding the signaling cascade. The value in human orphan GPCRs is that its utility as a research tool is enhanced in that by determining the location(s) of such receptors within the body, the GPCRs can be used to understand the role of these receptors in the human body before the endogenous ligand therefor is identified.

Other uses of the disclosed receptors will become apparent to those in the art based upon, *inter alia*, a review of this patent document.

EXAMPLES

The following examples are presented for purposes of elucidation, and not limitation, of the present invention. While specific nucleic acid and amino acid sequences are disclosed herein, those of ordinary skill in the art are credited with the ability to make minor modifications to these sequences while achieving the same or substantially similar results reported below. Unless otherwise indicated below, all nucleic acid sequences for the disclosed endogenous orphan human GPCRs have been sequenced and verified. For purposes of equivalent receptors, those of ordinary skill in the art will readily appreciate that conservative substitutions can be made to the disclosed sequences to obtain a functionally equivalent receptor.

Example 1

ENDOGENOUS HUMAN GPCRS

1. Identification of Human GPCRs

Several of the disclosed endogenous human GPCRs were identified based upon a review of the GenBank database information. While searching the database, the following cDNA clones were identified as evidenced below.

Disclosed	Accession	Complete DNA	Open Reading	Nucleic Acid	Amino
Human	Number	Sequence	Frame	SEQ.ID.	Acid
Orphan		(Base Pairs)	(Base Pairs)	NO.	SEQ.ID.
GPCRs					NO.

	hARE-3	AL033379	111,389 bp	1,260 bp	1	2
	hARE-4	AC006087	226,925 bp	1,119 bp	3	4
	hARE-5	AC006255	127,605 bp	1,104 bp	5	6
	hRUP3	AL035423	140,094 bp	1,005 bp	7	8
5	hRUP5	AC005849	169,144 bp	1,413 bp	9	10
	hRUP6	AC005871	218,807 bp	1,245 bp	11	12
	hRUP7	AC007922	158,858 bp	1,173 bp	13	14

Other disclosed endogenous human GPCRs were identified by conducting a BLAST search of EST database (dbest) using the following EST clones as query sequences. The 10 following EST clones identified were then used as a probe to screen a human genomic library.

	Disclosed	Query	EST Clone/	Open	Nucleic Acid	Amino Acid
	Human	(Sequence)	Accession No.	Reading	SEQ.ID.NO.	SEQ.ID.NO.
	Orphan		Identified	Frame		
15	GPCRs			(Base Pairs)		
	hGPCR27	Mouse	AA775870	1,125 bp	15	16
		GPCR27				
	hARE-1	TDAG	1689643	999 bp	17	18
			AI090920			
	hARE-2	GPCR27	68530	1,122 bp	19	20
			AA359504			
	hPPR1	Bovine	238667	1,053 bp	21	22
		PPR1	H67224			
20	hG2A	Mouse	<i>See Example 2(a),</i>	1,113 bp	23	24
		1179426	<i>below</i>			

	hCHN3	N.A.	EST 36581	1,113 bp	25	26
	hCHN4	TDAG	(full length) 1184934	1,077 bp	27	28
	hCHN6	N.A.	AA804531 EST 2134670	1,503 bp	29	30
	hCHN8	KIAA0001	(full length) EST 764455	1,029 bp	31	32
5	hCHN 9	1365839	EST 1541536	1,077 bp	33	34
	hCHN10	Mouse EST	Human 1365839	1,005 bp	35	36
	hRUP4	1365839 N.A.	AI307658	1,296 bp	37	38
		N.A. = "not applicable".				

2. Full Length Cloning

10 a. hG2A (Seq. Id. Nos. 23 & 24)

Mouse EST clone 1179426 was used to obtain a human genomic clone containing all but three amino acid hG2A coding sequences. The 5' end of this coding sequence was obtained by using 5'RACE™, and the template for PCR was Clontech's Human Spleen Marathon-ready™ cDNA. The disclosed human G2A was amplified by PCR using the G2A 15 cDNA specific primers for the first and second round PCR as shown in SEQ.ID.NO.: 39 and SEQ.ID.NO.:40 as follows:

5'-CTGTGTACAGCAGTTCGCAGAGTG-3' (SEQ.ID.NO.: 39; 1st round PCR)

5'-GAGTGCCAGGCAGAGCAGGTAGAC-3' (SEQ.ID.NO.: 40; second round PCR).

PCR was performed using Advantage™ GC Polymerase Kit (Clontech; manufacturing 20 instructions will be followed), at 94°C for 30 sec followed by 5 cycles of 94°C for 5 sec and 72°C for 4 min; and 30 cycles of 94° for 5 sec and 70° for 4 min. An approximate 1.3 Kb PCR fragment was purified from agarose gel, digested with Hind III and Xba I and cloned into the expression vector pRC/CMV2 (Invitrogen). The cloned-insert was sequenced using the T7 Sequenase™ kit (USB Amersham; manufacturer instructions will be followed) and

the sequence was compared with the presented sequence. Expression of the human G2A will be detected by probing an RNA dot blot (Clontech; manufacturer instructions will be followed) with the P³²-labeled fragment.

b. hCHN9 (Seq. Id. Nos. 33 & 34)

5 Sequencing of the EST clone 1541536 indicated that hCHN9 is a partial cDNA clone having only an initiation codon; *i.e.*, the termination codon was missing. When hCHN9 was used to "blast" against the data base (nr), the 3' sequence of hCHN9 was 100% homologous to the 5' untranslated region of the leukotriene B4 receptor cDNA, which contained a termination codon in the frame with hCHN9 coding sequence. To 10 determine whether the 5' untranslated region of LTB4R cDNA was the 3' sequence of hCHN9, PCR was performed using primers based upon the 5' sequence flanking the initiation codon found in hCHN9 and the 3' sequence around the termination codon found in the LTB4R 5' untranslated region. The 5' primer sequence utilized was as follows:

5'-CCCGAATTCCTGCTTGCTCCAGCTTGGCCC-3' (SEQ.ID.NO.: 41; sense) and

15 5'-TGTGGATCCTGCTGTCAAAGGTCCCATTCCGG-3' (SEQ.ID.NO.: 42; antisense).

PCR was performed using thymus cDNA as a template and rTth polymerase (Perkin Elmer) with the buffer system provided by the manufacturer, 0.25 uM of each primer, and 0.2 mM of each 4 nucleotides. The cycle condition was 30 cycles of 94°C for 1 min, 65°C for 1 min and 72 °C for 1 min and 10 sec. A 1.1kb fragment consistent with the predicted size was 20 obtained from PCR. This PCR fragment was subcloned into pCMV (*see below*) and sequenced (*see, SEQ.ID.NO.: 33*).

c. hRUP 4 (Seq. Id. Nos. 37 & 38)

The full length hRUP4 was cloned by RT-PCR with human brain cDNA (Clontech)

as templates:

5'-TCACAATGCTAGGTGTGGTC-3' (SEQ.ID.NO.: 43; sense) and

5'-TGCATAGACAATGGGATTACAG-3' (SEQ.ID.NO.: 44; antisense).

PCR was performed using TaqPlus™ Precision™ polymerase (Stratagene; manufacturing instructions will be followed) by the following cycles: 94°C for 2 min; 94°C 30 sec; 55°C for 30 sec, 72°C for 45 sec, and 72°C for 10 min. Cycles 2 through 4 were repeated 30 times.

The PCR products were separated on a 1% agarose gel and a 500 bp PCR fragment was isolated and cloned into the pCRII-TOPO vector (Invitrogen) and sequenced using the 10 T7 DNA Sequenase™ kit (Amsham) and the SP6/T7 primers (Stratagene). Sequence analysis revealed that the PCR fragment was indeed an alternatively spliced form of AI307658 having a continuous open reading frame with similarity to other GPCRs. The completed sequence of this PCR fragment was as follows:

5'-TCACAATGCTAGGTGTGGTCTGGCTGGTGGCAGTCATCGTAGGATCACCCATGTGGCAC
15 GTGCAACAACCTTGAGATCAAATATGACTTCTATATGAAAAGGAACACATCTGCTGCTTAGAA
GAGTGGACCAGCCCTGTGCACCAGAAGATCTACACCACCTTCATCCTTGTCATCCTCTTCTCTCC
TGCTCTTATGGTGATGCTTATCTGTACGTAAAAATTGGTTATGAACTTTGGATAAAGAAAAGA
GTTGGGGATGGTTCAGTGCTTCGAACTATTCATGAAAAAGAAATGTCCAAAATAGCCAGGAAG
AAGAAACGAGCTGTCATTATGATGGTGACAGTGGTGGCTCTCTTTGCTGTGTGCTGGGCACCA
20 TTCCATGTTGTCCATATGATGATTGAATACAGTAATTTTGAAAAGGAATATGATGATGTCACA
ATCAAGATGATTTTGCTATCGTGCAAATTATTGGATTTTCCAACTCCATCTGTAATCCCATTG
TCTATGCA-3' (SEQ.ID.NO.: 45)

Based on the above sequence, two sense oligonucleotide primer sets:

5'-CTGCTTAGAAGAGTGGACCAG-3' (SEQ.ID.NO.: 46; oligo 1),

25 5'-CTGTGCACCAGAAGATCTACAC-3' (SEQ.ID.NO.: 47; oligo 2)

and two antisense oligonucleotide primer sets:

5'-CAAGGATGAAGGTGGTGTAGA-3' (SEQ.ID.NO.: 48; oligo 3)

5'-GTGTAGATCTTCTGGTGCACAGG-3' (SEQ.ID.NO.: 49; oligo 4)

were used for 3'- and 5'-race PCR with a human brain Marathon-Ready™ cDNA (Clontech,

Cat# 7400-1) as template, according to manufacture's instructions. DNA fragments generated by the RACE PCR were cloned into the pCRII-TOPO™ vector (Invitrogen) and sequenced using the SP6/T7 primers (Stratagene) and some internal primers. The 3' RACE product contained a poly(A) tail and a completed open reading frame ending at a TAA stop codon. The 5' RACE product contained an incomplete 5' end; *i.e.*, the ATG initiation codon was not present.

Based on the new 5' sequence, oligo 3 and the following primer:

5'-GCAATGCAGGTCATAGTGAGC -3' (SEQ.ID.NO.: 50; oligo 5)

were used for the second round of 5' RACE PCR and the PCR products were analyzed as 10 above. A third round of 5' RACE PCR was carried out utilizing antisense primers:

5'-TGGAGCATGGTGACGGGAATGCAGAAG-3' (SEQ.ID.NO.: 51; oligo 6) and

5'-GTGATGAGCAGGTCAGCGCCAAG-3' (SEQ.ID.NO.: 52; oligo 7).

The sequence of the 5' RACE PCR products revealed the presence of the initiation codon ATG, and further round of 5' RACE PCR did not generate any more 5' sequence. The 15 completed 5' sequence was confirmed by RT-PCR using sense primer

5'-GCAATGCAGGCGCTTAACATTAC-3' (SEQ.ID.NO.: 53; oligo 8)

and oligo 4 as primers and sequence analysis of the 650 bp PCR product generated from human brain and heart cDNA templates (Clontech, Cat# 7404-1). The completed 3' sequence was confirmed by RT-PCR using oligo 2 and the following antisense primer:

20 5'-TTGGGTTACAATCTGAAGGGCA-3' (SEQ.ID.NO.: 54; oligo 9)

and sequence analysis of the 670 bp PCR product generated from human brain and heart cDNA templates. (Clontech, Cat# 7404-1).

d. hRUP5 (Seq. Id. Nos. 9 & 10)

The full length hRUP5 was cloned by RT-PCR using a sense primer upstream from

ATG, the initiation codon (SEQ.ID.NO.: 55), and an antisense primer containing TCA as the stop codon (SEQ.ID.NO.: 56), which had the following sequences:

5'-ACTCCGTGTCCAGCAGGACTCTG-3' (SEQ.ID.NO.:55)

5'-TGCGTGTTCTGGACCCCTCACGTG-3' (SEQ.ID.NO.: 56)

5 and human peripheral leukocyte cDNA (Clontech) as a template. Advantage cDNA polymerase (Clontech) was used for the amplification in a 50ul reaction by the following cycle with step 2 through step 4 repeated 30 times: 94°C for 30 sec; 94° for 15 sec; 69° for 40 sec; 72°C for 3 min; and 72°C for 6 min. A 1.4kb PCR fragment was isolated and cloned with the pCRII-TOPO™ vector (Invitrogen) and completely sequenced using the T7 DNA 10 Sequenase™ kit (Amsham). See, SEQ.ID.NO.: 9.

e. hRUP6 (Seq. Id. Nos. 11 & 12)

The full length hRUP6 was cloned by RT-PCR using primers:

5'-CAGGCCTTGGATTTTAATGTCAGGGATGG-3' (SEQ.ID.NO.: 57) and

5'-GGAGAGTCAGCTCTGAAAGAATTCAGG-3' (SEQ.ID.NO.: 58);

15 and human thymus Marathon-Ready™ cDNA (Clontech) as a template. Advantage cDNA polymerase (Clontech, according to manufacturer's instructions) was used for the amplification in a 50ul reaction by the following cycle: 94°C for 30sec; 94°C for 5 sec; 66°C for 40sec; 72°C for 2.5 sec and 72°C for 7 min. Cycles 2 through 4 were repeated 30 times. A 1.3 Kb PCR fragment was isolated and cloned into the pCRII-TOPO™ vector (Invitrogen) 20 and completely sequenced (see, SEQ.ID.NO.: 11) using the ABI Big Dye Terminator™ kit (P.E. Biosystem).

f. hRUP7 (Seq. Id. Nos. 13 & 14)

The full length RUP7 was cloned by RT-PCR using primers:

5'-TGATGTGATGCCAGATACTAATAGCAC-3' (SEQ.ID.NO.: 59; sense) and

5'-CCTGATTCATTAGGTGAGATTGAGAC-3' (SEQ.ID.NO.: 60; antisense)

and human peripheral leukocyte cDNA (Clontech) as a template. Advantage™ cDNA polymerase (Clontech) was used for the amplification in a 50 ul reaction by the following cycle with step 2 to step 4 repeated 30 times: 94°C for 2 minutes; 94°C for 15 seconds; 60°C for 20 seconds; 72°C for 2 minutes; 72°C for 10 minutes. A 1.25 Kb PCR fragment was isolated and cloned into the pCRII-TOPO™ vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator™ kit (P.E. Biosystem). See, SEQ.ID.NO.: 13.

g. hARE-5 (Seq. Id. Nos. 5 & 6)

The full length hARE-5 was cloned by PCR using the hARE5 specific primers 10 5'-CAGCGCAGGGTGAAGCCTGAGAGC-3' SEQ.ID.NO.: 69 (sense, 5' of initiation codon ATG) and 5'-GGCACCTGCTGTGACCTGTGCAGG-3' SEQ.ID.NO.:70 (antisense, 3' of stop codon TGA) and human genomic DNA as template. TaqPlus Precision™ DNA polymerase (Stratagene) was used for the amplification by the following cycle with step 2 to step 4 repeated 35 times: 96°C, 2 minutes; 96°C, 20 seconds; 58°C, 30 seconds; 72°C, 2 minutes; and 72°C, 10 minutes
15 A 1.1 Kb PCR fragment of predicated size was isolated and cloned into the pCRII-TOPO™ vector (Invitrogen) and completely sequenced (SEQ.ID.NO.:5) using the T7 DNA Sequenase™ kit (Amsham).

h. hARE-4 (Seq. Id. Nos.: 3 & 4)

The full length hARE-4 was cloned by PCR using the hARE-4 specific primers 5'-20 CTGGTGTGCTCCATGGCATCCC-3' SEQ.ID.NO.:67 (sense, 5' of initiation codon ATG) and 5'-GTAAGCCTCCAGAACGAGAGG-3' SEQ.ID.NO.: 68 (antisense, 3' of stop codon TGA) and human genomic DNA as template. Taq DNA polymerase (Stratagene) and 5% DMSO was used for the amplification by the following cycle with step 2 to step 3 repeated 35 times:

94°C, 3 minutes; 94°C, 30 seconds; 59°C, 2 minutes; 72°C, 10 minutes

A 1.12 Kb PCR fragment of predicated size was isolated and cloned into the pCRII-TOPO™ vector (Invitrogen) and completely sequenced (SEQ.ID.NO.:3) using the T7 DNA Sequenase™ kit (Amsham).

5 **i. hARE-3 (Seq.Id.Nos.: 1 & 2)**

The full length hARE-3 was cloned by PCR using the hARE-3 specific primers 5'-gatcaagcttCCATCCTACTGAAACCATGGTC-3' SEQ.ID.NO.:65 (sense, lower case nucleotides represent Hind III overhang, **ATG** as initiation codon) and 5'-gatcagatctCAGTTCCAATATTCACACCACCGTC-3' SEQ.ID.NO.:66 (antisense, lower case 10 nucleotides represent Xba I overhang, **TCA** as stop codon) and human genomic DNA as template. TaqPlus Precision™ DNA polymerase (Stratagene) was used for the amplification by the following cycle with step 2 to step 4 repeated 35 times: 94°C, 3 minutes; 94°C, 1 minute; 55°C, 1 minute; 72°C, 2 minutes; 72°C, 10 minutes.

A 1.3 Kb PCR fragment of predicated size was isolated and digested with Hind III 15 and Xba I, cloned into the pRC/CMV2 vector (Invitrogen) at the Hind III and Xba I sites and completely sequenced (SEQ.ID.NO.:1) using the T7 DNA Sequenase™ kit (Amsham).

j. hRUP3 (Seq. Id. Nos.:7 & 8)

The full length hRUP3 was cloned by PCR using the hRUP3 specific primers 5'-GTCCTGCCACTTCGAGACATGG-3' SEQ.ID.NO.:71 (sense, **ATG** as initiation codon) and 5'-20 GAAACTTCTCTGCCCTTACCGTC-3' SEQ.ID.NO.:72 (antisense, 3' of stop codon TAA) and human genomic DNA as template. TaqPlus Precision™ DNA polymerase (Stratagene) was used for the amplification by the following cycle with step 2 to step 4 repeated 35 times: 94°C, 3 minutes; 94°C, 1 minute; 58°C, 1 minute; 72°C, 2 minutes; 72°C, 10 minutes

A 1.0 Kb PCR fragment of predicated size was isolated and cloned into the pCRII-TOPO™ vector (Invitrogen) and completely sequenced (SEQ.ID.NO.: 7) using the T7 DNA sequenase kit (Amsham).

Example 2

5 RECEPTOR EXPRESSION

Although a variety of cells are available to the art for the expression of proteins, it is most preferred that mammalian cells be utilized. The primary reason for this is predicated upon practicalities, *i.e.*, utilization of, *e.g.*, yeast cells for the expression of a GPCR, while possible, introduces into the protocol a non-mammalian cell which may not (indeed, in the 10 case of yeast, does not) include the receptor-coupling, genetic-mechanism and secretary pathways that have evolved for mammalian systems - thus, results obtained in non-mammalian cells, while of potential use, are not as preferred as that obtained from mammalian cells. Of the mammalian cells, COS-7, 293 and 293T cells are particularly preferred, although the specific mammalian cell utilized can be predicated upon the particular 15 needs of the artisan. The general procedure for expression of the disclosed GPCRs is as follows.

On day one, 1×10^7 293T cells per 150mm plate were plated out. On day two, two reaction tubes will be prepared (the proportions to follow for each tube are per plate): tube A will be prepared by mixing 20μg DNA (*e.g.*, pCMV vector; pCMV vector with receptor 20 cDNA, etc.) in 1.2ml serum free DMEM (Irvine Scientific, Irvine, CA); tube B will be prepared by mixing 120μl lipofectamine (Gibco BRL) in 1.2ml serum free DMEM. Tubes A and B are admixed by inversions (several times), followed by incubation at room temperature for 30-45min. The admixture can be referred to as the "transfection mixture". Plated 293T cells are washed with 1XPBS, followed by addition of 10ml serum free DMEM.

2.4ml of the transfection mixture will then be added to the cells, followed by incubation for 4hrs at 37°C/5% CO₂. The transfection mixture was then be removed by aspiration, followed by the addition of 25ml of DMEM/10% Fetal Bovine Serum. Cells will then be incubated at 37°C/5% CO₂. After 72hr incubation, cells can then be harvested and utilized for analysis.

5 Example 3

TISSUE DISTRIBUTION OF THE DISCLOSED HUMAN GPCRS

Several approaches can be used for determination of the tissue distribution of the GPCRs disclosed herein.

1. Dot-Blot Analysis

10 Using a commercially available human-tissue dot-blot format, endogenous orphan GPCRs were probed for a determination of the areas where such receptors are localized. cDNA fragments from the GPCRs of Example 1 (radiolabelled) were (or can be) used as the probe: radiolabeled probe was (or can be) generated using the complete receptor cDNA (excised from the vector) using a Prime-It II™ Random Primer Labeling Kit (Stratagene, 15 #300385), according to manufacturer's instructions. A human RNA Master Blot™ (Clontech, #7770-1) was hybridized with the endogenous human GPCR radiolabeled probe and washed under stringent conditions according manufacturer's instructions. The blot was exposed to Kodak BioMax™ Autoradiography film overnight at -80°C. Results are summarized for several receptors in Table B and C (*see* Figures 1A and 1B for a grid 20 identifying the various tissues and their locations, respectively). Exemplary dot-blot results are provided in Figure 2A and 2B for results derived using hCHN3 and hCHN8, respectively.

TABLE B

ORPHAN GPCR

Tissue Distribution
(highest levels, relative to other tissues in the dot-blot)

	hGPCR27	Fetal brain, Putamen, Pituitary gland, Caudate nucleus
	hARE-1	Spleen, Peripheral leukocytes, Fetal spleen
	hPPR1	Pituitary gland, Heart, salivary gland, Small intestine, Testis
	hRUP3	Pancreas
5	hCHN3	Fetal brain, Putamen, Occipital cortex
	hCHN9	Pancreas, Small intestine, Liver
	hCHN10	Kidney, Thyroid

TABLE C

	ORPHAN GPCR	Tissue Distribution (highest levels, relative to other tissues in the dot-blot)
10	hARE-3	Cerebellum left, Cerebellum right, Testis, Accumbens
	hGPCR3	Corpus collusum, Caudate nucleus, Liver, Heart, Inter-Ventricular Septum
	hARE-2	Cerebellum left, Cerebellum right, Substantia
	hCHN8	Cerebellum left, Cerebellum right, Kidney, Lung

2. RT-PCR

15 a. hRUP3

To ascertain the tissue distribution of hRUP3 mRNA, RT-PCR was performed using hRUP3-specific primers and human multiple tissue cDNA panels (MTC, Clontech) as templates. Taq DNA polymerase (Stratagene) was utilized for the PCR reaction, using the following reaction cycles in a 40ul reaction: 94°C for 2 min; 94°C for 15 sec; 55°C for 30 sec; 72°C for 1 min; 72°C, for 10 min. Primers were as follows:

5'-GACAGGTACCTTGCCATCAAG-3' (SEQ.ID.NO.: 61; sense)

5'-CTGCACAATGCCAGTGATAAGG-3' (SEQ.ID.NO.: 62; antisense).

20ul of the reaction was loaded onto a 1% agarose gel; results are set forth in Figure 3.

As is supported by the data of Figure 3, of the 16 human tissues in the cDNA panel utilized (brain, colon, heart, kidney, lung, ovary, pancreas, placenta, prostate, skeleton, small intestine, spleen, testis, thymus leukocyte, and liver) a single hRUP3 band is evident only from the pancreas. Additional comparative analysis of the protein sequence of hRUP3 with 5 other GPCRs suggest that hRUP3 is related to GPCRs having small molecule endogenous ligand such that it is predicted that the endogenous ligand for hRUP3 is a small molecule.

b. hRUP4

RT-PCR was performed using hRUP4 oligo's 8 and 4 as primers and the human multiple tissue cDNA panels (MTC, Clontech) as templates. Taq DNA polymerase 10 (Stratagene) was used for the amplification in a 40ul reaction by the following cycles: 94°C for 30 seconds, 94°C for 10 seconds, 55°C for 30 seconds, 72°C for 2 minutes, and 72°C for 5 minutes with cycles 2 through 4 repeated 30 times.

20 μ l of the reaction were loaded on a 1% agarose gel to analyze the RT-PCR products, and hRUP4 mRNA was found expressed in many human tissues, with the strongest 15 expression in heart and kidney. (see, Figure 4). To confirm the authenticity of the PCR fragments, a 300 bp fragment derived from the 5' end of hRUP4 was used as a probe for the Southern Blot analysis. The probe was labeled with 32 P-dCTP using the Prime-It II™ Random Primer Labeling Kit (Stratagene) and purified using the ProbeQuant™ G-50 micro columns (Amersham). Hybridization was done overnight at 42° C following a 12 hr pre-20 hybridization. The blot was finally washed at 65°C with 0.1 x SSC. The Southern blot did confirm the PCR fragments as hRUP4.

c. hRUP5

RT-PCR was performed using the following hRUP5 specific primers:

5'-CTGACTTCTTGTTCCTGGCAGCAGCGG-3' (SEQ.ID.NO.: 63; sense)

5'-AGACCAGCCAGGGCAGCTGAAGAGTG-3' (SEQ.ID.NO.: 64; antisense)

and the human multiple tissue cDNA panels (MTC, Clontech) as templates. Taq DNA polymerase (Stratagene) was used for the amplification in a 40ul reaction by the following cycles: 94°C for 30 sec, 94°C for 10 sec, 62°C for 1.5 min, 72°C for 5 min, and with cycles 2 through 3 repeated 30 times. 20 µl of the reaction were loaded on a 1.5% agarose gel to analyze the RT-PCR products, and hRUP5 mRNA was found expressed only in the peripheral blood leukocytes (*data not shown*).

10 **d. hRUP6**

RT-PCR was applied to confirm the expression and to determine the tissue distribution of hRUP6. Oligonucleotides used, based on an alignment of AC005871 and GPR66 segments, had the following sequences:

5'-CCAACACCAGCATCCATGGCATCAAG-3' (SEQ.ID.NO.: 73; sense),

15 5'-GGAGAGTCAGCTCTGAAAGAATTCAGG-3' (SEQ.ID.NO.: 74; antisense)

and the human multiple tissue cDNA panels (MTC, Clontech) were used as templates.

PCR was performed using TaqPlus Precision™ polymerase (Stratagene; manufacturing instructions will be followed) in a 40ul reaction by the following cycles: 94°C for 30 sec; 94°C 5 sec; 66°C for 40 sec, 72°C for 2.5 min, and 72°C for 7 min. Cycles 2 through 4
20 were repeated 30 times.

20 ul of the reaction were loaded on a 1.2% agarose gel to analyze the RT-PCR products, and a specific 760bp DNA fragment representing hRUP6 was expressed predominantly in the thymus and with less expression in the heart, kidney, lung, prostate small intestine and testis. (*see, Figure 5*).

It is intended that each of the patents, applications, and printed publications mentioned in this patent document be hereby incorporated by reference in their entirety.

As those skilled in the art will appreciate, numerous changes and modifications may be made to the preferred embodiments of the invention without departing from the spirit of the invention. It is intended that all such variations fall within the scope of the invention and the claims that follow.

Although a variety of Vectors are available to those in the art, for purposes of utilization for both endogenous and non-endogenous human GPCRs, it is most preferred that the Vector utilized be pCMV. This vector was deposited with the American Type Culture Collection (ATCC) on October 13, 1998 (10801 University Blvd., Manassas, VA 20110-2209 USA) under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. The DNA was tested by the ATCC and determined to be. The ATCC has assigned the following deposit number to pCMV: ATCC #203351.